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ORIGINAL INVESTIGATION

Exhibit B
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Self-administration of cocaine analogs by rats

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Abstract *Rationale*: A novel scheme for the synthesis of cocaine analogs from vinylcarbenoid precursors has made available compounds that have a diverse range of affinities for the DA and 5-HT transporters. These compounds were used to explore the relationship between their biochemical properties and their reinforcing effects. *Objectives*: The objective was to assess the reinforcing efficacy of selected cocaine analogs and compare the results with their selectivity in binding to DA and 5-HT transporters. *Methods*: Rats were prepared with chronically indwelling intravenous cannulae and trained to self-administer cocaine on a progressive ratio (PR) schedule. A range of doses of seven cocaine analogs were substituted for cocaine in separate groups of animals. *Results*: The results demonstrate a wide range of reinforcing efficacies and potencies among the seven selected drugs. Four tropane analogs (WF-11, WF-23, WF-24, WF-55) were found to support self-administration behavior on a PR schedule while three did not (WF-31, WF-54 and WF-60). The DA/5-HT selectivity ratio was found to be a better predictor of self-administration behavior than affinity at the DA transporter alone. *Conclusion*: These data suggest that drugs with a higher affinity for the DA versus the 5-HT transporter are more likely to be self-administered.

Key words Cocaine · Tropane analogs · Self-administration · Progressive-ratio schedule

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Introduction

Cocaine produces a number of behavioral reactions in humans, including elevated mood, psychomotor stimulation, heightened sexual interest and decreased appetite (Jaffe 1993); some or all of these may contribute to the well know abuse liability of cocaine. Cocaine can also elicit anxious and/or psychotic-like symptoms which may complicate the manifestations of addictive behavior. The neurobiology underlying these complex behavioral responses is only beginning to be understood. Cocaine has been shown to bind to dopamine (DA), norepinephrine (NE) and serotonin (5-HT) transporters and inhibit the reuptake of these neurotransmitters into their respective presynaptic terminals (Heikkila et al. 1975; Moore et al. 1977). Through this action, cocaine increases the extracellular monoamine concentrations and can therefore be classified as a non-selective indirect agonist at DA, NE and 5-HT receptors. Since these transmitter systems have widespread terminal projections, cocaine presumably produces physiological actions throughout the nervous system. Despite these widespread effects, progress has been made in ascribing distinct behavioral reactions to discrete monoamine fluctuations within anatomically specific regions.

The preponderance of data indicates that DA plays a critical role in cocaine self-administration (Woolverton and Johnson 1992; Koob and Le Moal 1997). Studies designed to associate the behavioral effects of cocaine with specific anatomical and molecular sites of action have involved a wide variety of research strategies, each with their own limitations. Analyses of the effects of neurotoxic lesions on IV drug self-administration have provided evidence linking the meso-limbic and meso-cortical DA projections with the expression of the acute reinforcing effects of cocaine (Roberts et al. 1977; Schenk et al. 1991; Koob 1992; Caine and Koob 1994; McGregor et al. 1996), while ascending 5-HT systems appear to play a lesser role or even antagonize the reinforcing effects of psychomotor stimulants (Leccese and Lyness 1984; Loh and Roberts 1990). The lesion strategy, however, is com-

promised by the effects of non-specific damage and variable time courses of degenerative and compensatory changes. Systemic pretreatments with specific receptor antagonists have helped identify receptor subtypes that modify cocaine self-administration (De Wit and Wise 1977; Roberts and Vickers 1984; Woolverton and Virus 1990; Britton et al. 1991), although this approach suffers from drawbacks associated with drug-drug interactions, including issues related to differing time courses and metabolism.

A complimentary strategy to these approaches has been to modify systematically the chemical structure of cocaine and to assess the changes in biochemical and behavioral activity. Correlations between binding affinity and reinforcing efficacy can thereby be explored (e.g. Ritz et al. 1987). A number of novel cocaine derivatives have been prepared and evaluated for biological activity (Madras et al. 1989; Abraham et al. 1992; Boja et al. 1994; Carroll et al. 1995; Kozikowski et al. 1995; Fleckenstein et al. 1996; Agoston et al. 1997; Chang et al. 1997; Lomenzo et al. 1997; Meltzer et al. 1997). (–)-Cocaine has been the starting material for most of these compounds and this has limited the types of structural modifications that can be accomplished. Consequently, alternative synthetic strategies to cocaine derivatives have been explored (Chen and Meltzer 1997; Kozikowski et al. 1998). A particularly attractive synthetic strategy was developed by Davies et al. (1993, 1994) using vinylcarbenoid precursors. Over 130 new tropane derivatives have been prepared by this approach and all of these compounds have been tested in binding and uptake assays. Non-selective but highly potent compounds have been identified as well as compounds that show selectivity for either the DA or 5-HT transporters (Davies et al. 1996).

In the present experiments, seven tropane analogs that represent a range of monoamine selectivities were examined for their ability to support self-administration in rats. Each of these compounds was tested for self-administration using a progressive ratio (PR) schedule, which allows for relative assessment of both potency and reinforcing efficacy (Richardson and Roberts 1996). The PR schedule was first developed by Hodos (1961) to examine the reinforcing effects of sweetened milk solutions, and has since been applied in various implementations to the study of self-administration behavior in primates (Griffiths et al. 1975; Hoffmeister 1979; Woolverton 1995), dogs (Risner and Silcox 1981) and rodents (Roberts 1989; Depoortere et al. 1993). The schedule has been shown to be particularly useful in evaluating psychomotor stimulant drugs such as TCP, GBR 12909, meth- and dextro-amphetamine (Roberts 1993; French et al. 1995; Richardson and Roberts 1996). The dependent measure, breaking point, is sensitive to pharmacological, hormonal and neurotoxic manipulations (Roberts and Goeders 1989; Roberts et al. 1989; Loh and Roberts 1990; McGregor and Roberts 1993). A critique of the limitations of the PR schedule and a comparison with FR schedules has recently been published (Arnold and Roberts 1997).

Materials and methods

Subjects

Male Wistar rats (Charles River Farms, Quebec, Canada) weighing 275–300 g at the start of the experiment served as subjects. Upon arrival from the supplier, rats were housed two per cage and maintained on a 12-h reverse light/dark cycle (lights on at 3 p.m.) with food and water available ad libitum in the home cage for at least 1 week. Rats were then food deprived for 24 h and trained to press a response lever under a fixed-ratio (FR) 1 schedule of food presentation. Thereafter food was again available ad libitum for the remainder of the experiment. All research was approved by the Carleton University Animal Care Committee and conducted according to the Guide for the Care and Use of Laboratory Animals as promulgated by the National Institutes of Health and the Canadian Council on Animal Care.

Self-administration

Rats were anaesthetized with pentobarbital (60 mg/kg and supplemented as needed), and implanted with a chronically indwelling Silastic jugular cannula that exited through the skin on the dorsal surface in the region of the scapulae (Roberts and Goeders 1989). Following cannulation, each rat was singly housed in a 25×25×25 cm experimental chamber. The cannula was attached through a protective spring to a counter-balanced fluid swivel that allowed free movement within the experimental chamber. The cannula assembly was connected to a Razel syringe pump equipped with a 5 rpm motor.

Initially, rats were given access to a lever during daily 6-h sessions. Each operant response activated an injection pump that delivered 0.25 mg/injection cocaine HCl (0.1 ml/5 s). This is equivalent to an average unit injection dose of 0.75 mg/kg per injection. Concurrent with the start of the injection, a stimulus light was activated that signaled a 20-s post-infusion time-out period during which time responses produced no programmed consequence.

Rats that had demonstrated a constant rate of operant responding on the FR1 schedule (>30 injections/5 h and regular post-infusion pauses assessed by visual inspection of the data) were given access to cocaine (1.5 mg/kg per injection) on a PR schedule. Each rat received one "priming" injection at the start of each daily session. The next reinforcement was contingent on a single operant response; thereafter the number of responses required to obtain subsequent infusions was incremented through the following progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603. The equation used to calculate the series was:

$$\text{ratio}=(5\times e^{(0.2\times\text{infusion number})})-5$$

This implementation has been described in detail elsewhere (Richardson and Roberts 1996).

Seven separate groups of rats were used to test the reinforcing effects of the selected tropane analogs. Rats were trained to self-administer cocaine on the PR schedule until a stable pattern had developed (defined as 3 consecutive days of cocaine self-administration with break points within a range of three). When cocaine self-administration was stable, a tropane analog was substituted for cocaine for four consecutive test sessions. On the fifth session, cocaine self-administration was reassessed. If the IV cannula remained patent and a stable baseline pattern of cocaine self-administration could be re-established, a different dose of the selected tropane was evaluated. The order of testing for each dose was counterbalanced within the group (n=5–7 per dose). The following tropanes (and dosages) were evaluated: WF-11 (vehicle, 0.03, 0.1, 0.17, 0.3 mg/kg per injection); WF-23 (vehicle, 0.003, 0.01, 0.03, 0.056, 0.1 mg/kg per injection); WF-24 (0.1, 0.3, 0.56, 1.0, 1.7 mg/kg per injection); WF-31 (vehicle, 0.17, 0.3, 0.56); WF-54 (vehicle, 0.03, 0.3, 0.1, 1.7); WF-55 (vehicle, 0.003, 0.03, 0.1, 0.3); and WF-60 (vehicle, 0.03, 0.1, 0.3, 1.0). Selection of doses

was based on preliminary behavioral data. In the case of WF-24, WF-31, WF-54 and WF-60, doses were escalated to the limits of solubility in saline at a volume of 0.1 ml/injection.

The parameters chosen for the PR schedule were derived from previous cocaine self-administration experiments (Roberts et al. 1989; Richardson et al. 1993; McGregor et al. 1994). The minimum session length was 6 h. The session was extended until a period of non-reinforced responding was observed. This period was always at least 3 times the average inter-infusion interval, or to a maximum session length of 22 h (the maximum permitted by the Carleton University Animal Care Committee protocol).

Statistics

Break point was defined as the final ratio completed during the session. Since final ratios are derived from an escalating (exponential) function, the data must be log transformed to an interval scale prior to analysis. One such transformation converts the final ratio values into the number of injections in the series. Therefore, the number of infusions self-administered was used in all statistical analyses. For clarity, the corresponding final ratios are shown on all figures. Data were subjected to a repeated measures ANOVA, with Days as a within-group dependent variable. Newman-Keuls test was used to compare break points from day 4 of substitution of individual doses with data from vehicle substitution. Pearson's product moment correlations were used to evaluate the relationship between binding affinity and maximum break point for each drug.

Results

Three tropane compounds failed to support self-administration behavior on the PR schedule at the dosages tested. Figure 1 shows that break points for WF-31, WF-54 and WF-60 declined during the 4 days of substitution testing and were similar to vehicle substitution tests. None of the doses was significantly different from saline on the fourth substitution session. Re-introduction of cocaine resulted in reinstatement of regularly patterned self-administration, although in some cases the break points on cocaine were suppressed for a day or two before returning to pre-test values.

Figure 2 shows the break points for the four tropane analogs (WF-11, WF-23, WF-24, WF-55) that were found to support self-administration behavior on a PR schedule. Substitution of these tropane analogs produced dose dependent effects on break point (see below) that were significantly higher than saline break points.

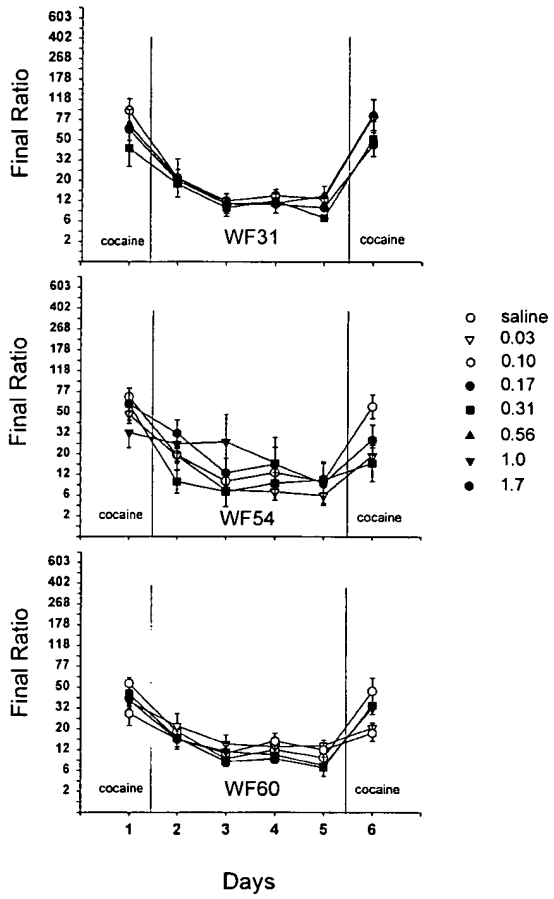


Fig. 1 Effect of substitution of WF-31, WF-54 or WF-60 on final ratios established on a PR schedule of reinforcement. After rats had demonstrated a stable baseline pattern of cocaine self-administration (0.75 mg/kg per injection), various doses of WF-31, WF-54, WF-60 or saline were substituted during four consecutive daily sessions. No statistically significant differences were observed compared to saline substitution. Data represent the mean (±SEM) number of injections self-administered during the session (n=5–7/group). For clarity, the corresponding final ratios are shown on the *abscissa*

WF-11 (PTT) self-administration

Figure 2 shows the effect of substituting various doses of WF-11 for cocaine on break points established on a PR schedule. ANOVA revealed a significant effect of Dose [$F(4,24)=35.58$; $P<0.001$]. At the highest dose of WF-11 tested (0.3 mg/kg per injection), rats self-administered a mean of 21.6 injections on day 4 of substitution, which corresponds to a mean final ratio between 382 and 402. WF-11 supported self-administration behavior in all animals previously trained to self-administer cocaine (0.3 mg/kg per injection : range 19–27). Figure 3 illustrates a representative cumulative response record of WF-11 self-administration on a PR schedule. During the early part of the session, infusions were regularly spaced. Each infusion was followed by a relatively consistent post-reinforcement pause, after which the animal responded until the requirement for the next infusion was met. At some point in the session, responding ceased, except for the very occasional response. The response pattern is similar to that previously described for cocaine except that the post-reinforcement pauses are much longer (see Fig. 3). Note that in the examples shown in Fig. 3, the final ratio established for the most effective dose of WF11 (0.3 mg/kg per injection) was not reached until 15 h into the session, whereas the final ratio for the most effective dose of cocaine (1.5 mg/kg per injection) is reached within 2 h.

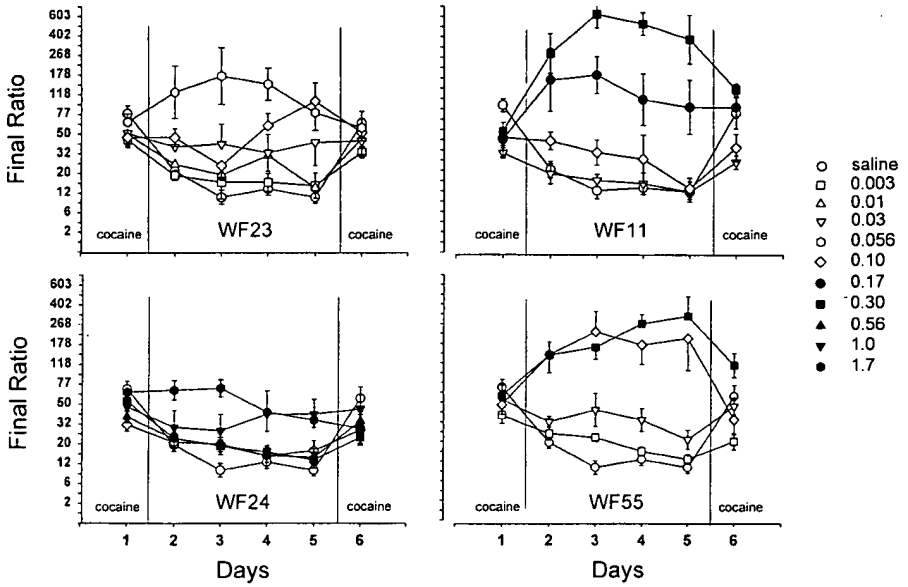


Fig. 2 Effect of substitution of WF-11, WF-23, WF-24 or WF55 on final ratios established on a PR schedule of reinforcement. After rats had demonstrated a stable baseline pattern of cocaine self-administration (0.75 mg/kg per injection), various doses of WF-11, WF-23, WF-24 or WF55 or saline (n=5–7/group) were substituted during four consecutive daily sessions. Each of the four tropane analogs was found to support self-administration on a PR schedule in a dose dependent manner. Data represent the mean (±SEM) number of injections self-administered during the session (n=5–7/group). For clarity, the corresponding final ratios are shown on the *abscissa*

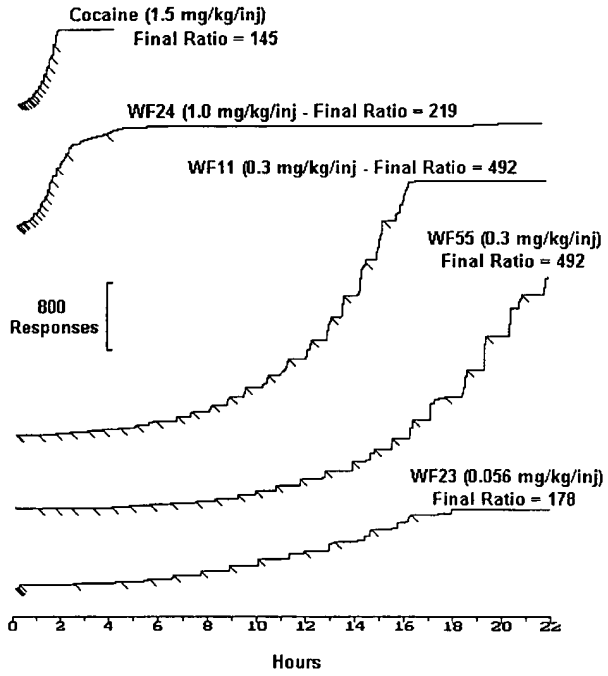


Fig. 3 Patterns of self-administration of various tropane analogs reinforced on a PR schedule. Each line represents a cumulative record. Upward excursions of the line represent responses and downward tick marks indicate the time of each injection. The response pattern is similar for each drug, except for the length of the post-reinforcement pause. During the early part of the session infusions are regularly spaced. Each infusion is followed by a relatively consistent post-reinforcement pause, after which the animal responds until the requirement for the next infusion is reached. At some point in the session, responding ceased, except for the very occasional response (see Fig. 4). Different groups of animals were used to test each drug

WF-23 self-administration

Figure 2 shows the effect of substituting various doses of WF-23 for cocaine on break points established on a PR schedule. ANOVA revealed a significant effect of Dose [$F(5,37)=4.45$; $P<0.05$] on break points established on a PR schedule. The three highest doses tested (0.03, 0.056 and 0.1 mg/kg per injection) supported break points significantly higher than saline. Figure 3 illustrates a representative cumulative response record of WF-23 self-administration on a PR schedule. Typical of this drug, the inter-infusion intervals were extremely long, upwards of 90 min at a dose of 0.056 mg/kg per injection. Note that in this example, responding was distinctly patterned, although only 18 injections were self-administered. Failure to cease responding prior to the end of the session was seen in three of seven animals tested at the highest dose. Truncating the session at 22 h therefore caused a significant underestimate of the mean break point.

WF-24 self-administration

Figure 2 shows the effect of substituting various doses of WF-24 for cocaine on break points established on a PR schedule. ANOVA revealed a significant effect of Dose [$F(5,33)=3.48$; $P<0.05$] on break points. Self-administration of WF-24 was found to be variable. Break point values at the highest dose tested ranged from final ratios of 9 and 145 corresponding to a total of 5–17 injections/session. Inter-infusion intervals were relatively short compared to the other tropanes reported here. An example of a cumulative record from an animal that responded to relatively high break points is shown in Fig. 3. Final ratios were reached within the 5-h test session; extending the session had no effect on break points.

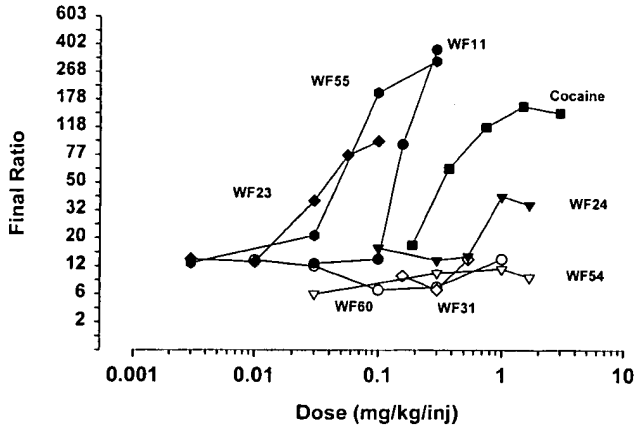


Fig. 4 Responding on a PR schedule reinforced by various doses of seven tropane analogs. Rats were first trained to respond for cocaine reinforcement (0.75 mg/kg per injection), then various doses of WF11, WF23, WF24, WF31, WF54, WF55 or WF60 were substituted for four consecutive daily sessions. Data represent the mean final ratio established on the fourth substitution day. WF60, WF31 and WF54 were not significantly different from saline substitution. WF23, WF55, WF11 and WF24 showed statistically significant dose-dependent self-administration

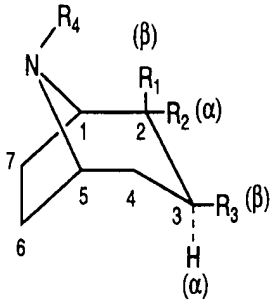
WF-55 self-administration

Figure 2 shows the effect of substituting various doses of WF-55 for cocaine on break points established on a PR schedule. ANOVA revealed a significant effect of Dose [$F(4,27)=22.35$; $P<0.001$] on break points. The three highest doses (0.03, 0.1 and 0.3 mg/kg per injection) supported self-administration behavior above saline substitution levels. Figure 3 illustrates a representative cumulative response record of WF-55 self-administration on a PR schedule. WF-55 self-administration was characterized by a regular pattern consistent with previous descriptions of psychostimulant self-administration on a PR schedule. Note that in this example responding was distinctly patterned and continued to the end of the 22-h session. Failure to cease responding prior to the end of the session was seen in two of seven animals tested at the highest dose. Truncating the session at 22 h therefore caused a significant underestimate of the mean break point.

Figure 4 illustrates the dose-response curves for the seven tropane analogs tested. The points represent the average break point achieved on day 4 of tropane substitution. A cocaine dose-response curve is included for comparison.

Table 1 Binding displacement and uptake inhibition data for selected cocaine analogs. Binding to DA transporters ([^{125}I]RTI-55 binding) and 5-HT transporters ([^{3}H]paroxetine binding) was determined in brain membranes as described by Bennett et al. (1995). By convention, data are expressed as IC_{50} values at dopamine transporters because of multiple binding sites in these assays, and K_i values at 5-HT transport sites. Data for cocaine, WF-11, WF-23, WF24, WF31 are taken from Bennett et al. (1995). DA/5-HT selectivity was calculated as ratios from data in the *Binding* and *Uptake* columns. Values >1 indicate a relatively greater specificity for DA versus 5-HT. Break points represent the maximum mean values from a range of doses (see Fig. 4)

Self-administration of cocaine analogs



Compound					Binding displacement		Uptake inhibition		DA/5-HT selectivity		Break point
	R1	R2	R3	R4	DA IC_{50} (nM)	5-HT K_i (nM)	[^{3}H]DA IC_{50} (nM)	[^{3}H]5HT IC_{50} (nM)	Binding	Uptake	
Cocaine	$COOCH_3$	H	COOPh	Me	173	302	150	480	1.75	3.2	17.5
WF-11	$COCH_2CH_3$	H	Ph-CH_3	Me	8.2	131	3.2	448	16	141	21.6
WF-23	$COCH_2CH_3$	H	2-napthyl	Me	0.12	0.4	0.65	0.32	3.25	0.49	15
WF-24	H	$COCH_2CH_3$	2-napthyl	Me	2.5	16.4	5.9	5.8	6.4	4.92	11
WF-31	$COCH_2CH_3$	H	$PhCH(CH_3)_2$	Me	436	35.8	669	49	0.08	0.07	6.5
WF-54	$COCH_2CH_3$	H	Ph-methyl ethenyl	Me	7.2	0.8	40.8	3.98	0.11	0.1	5.8
WF-55	$COCH_2CH_3$	H	Ph-ethenyl	Me	1.0	3.6	0.9	37.6	3.78	41.7	20.7
WF-60	$COCH_2CH_3$	H	Ph-methyl ethenyl	H	15.8	0.1	48.6	1.98	0.006	0.04	6.4

Discussion

The present experiments evaluated the reinforcing properties of seven novel tropane analogs. The potency and efficacy of each drug to support self-administration behavior was evaluated using a progressive-ratio schedule of reinforcement. The results demonstrate a wide range of reinforcing efficacies and potencies among the selected drugs.

Table 1 summarizes the potencies of the selected cocaine analogs in binding to DA transporters ([^{125}I]RTI-55 binding) and 5-HT transporters ([^{3}H]paroxetine binding) and inhibition of [^{3}H]DA and [^{3}H]5-HT uptake (Bennett et al. 1995). The formulae refer to the following general structure of tropanes, with the various R groups positioned as in Table 1. In this series of analogs, both ester linkages have been eliminated, thus increasing potential metabolic stability. All of these novel tropanes contain (1) an alkyl ketone moiety in the 2-position (the most active tropanes have these alkyl ketones in the α position) and (2) a substituted aryl (either phenyl or naphthyl) moiety at the 3-position. A change in selectivity for DA or 5-HT transporters can be accomplished through manipulation of the moiety at position R_4 (see Table 1). DA selectivity can be conferred by an *N*-methyl group at R_4; whereas if R_4 is an H, then the tropane is likely to be 5-HT selective (Boja et al. 1994). The seven analogs were selected so as to represent a continuum of affinities at the DA and 5-HT transporters. Drugs ranged from highly specific for the 5-HT transporter (e.g. WF-60) to the relatively specific DA compound, WF-11.

Ritz et al. (1987) reported that the potencies of cocaine-like compounds in self-administration studies correlate with their potencies in inhibiting [^{3}H]-mazindol binding to the DA transporter, but not to their potencies in binding to a wide range of other pre- and post-synaptic binding sites. These data were consistent with an extensive literature indicating a primary role for DA in the reinforcing effects of psychostimulant drugs. The present data set offers an opportunity to examine similar relationships between reinforcing efficacy, as measured by maximum break point using standardized procedures, and in vitro binding affinity.

While this analysis should be considered preliminary since only seven compounds have been tested thus far, the results are generally consistent with the hypothesis that drugs having a high affinity for the DA transporter have appreciable reinforcing efficacies; however, the present data illustrate that this relationship may not be a simple one. Surprisingly, the correlation of break point with binding affinity at the DA transporter was relatively poor (r=0.31). It appears that high affinity for the DA transporter is not sufficient to impart a significant level of reinforcing efficacy. Six of the seven compounds studied have higher affinities for the DAT than cocaine yet only three were found to be self-administered to high break points. For example, WF-54 is 24 times more potent at the DA transporter than cocaine and approximately equipotent to WF-11 but was found not to be self-administered by rats. Note also, that three drugs which displayed approximately the same affinity for the DA transporter (WF-11, WF-54 and WF-60) had very disparate reinforcing effects. While other factors such as duration of action, bioavailability, lipophilicity, absorption etc. might account for such discrepancies, the present data indicate that DA/5-HT selectivity may be an important factor. Regardless of whether the binding or the reuptake data were used to calculate DA/5-HT selectivity, the correlation with break point was identical (r=0.67). Our preliminary speculation is that, in addition to having affinity for the DA transporter, drugs must also have a lower affinity for the 5-HT versus the DA transporter in order to be self-administered. This is in accord with the Ritz et al. (1987) report of a small negative correlation between potency in self-administration studies and an inhibition of serotonin uptake.

A substantial literature suggests that DA and 5-HT are both involved in cocaine's actions but in fundamentally different and perhaps opposite ways (Cunningham et al. 1995). The requirement for a preferential effect on DA over 5-HT transporters is consistent with the idea that serotonin has a negative impact on psychostimulant reinforcement. Removal of ascending 5-HT fiber systems appears to augment cocaine and amphetamine self-administration (Lyness et al. 1980; Loh and Roberts 1990), and drugs that promote serotonin function may attenuate cocaine reinforcement (Smith et al. 1986; Carroll et al. 1990; Yu et al. 1990; Richardson and Roberts 1991; McGregor et al. 1993). None of the 5-HT specific drugs tested here would appear to have significant reinforcing efficacy.

WF-11 (also described as PTT) was chosen for evaluation because of its moderate (16:1) selectivity for the DA versus the 5-HT transporter. WF-11 is approximately 10 times more potent than cocaine in vivo, and produces long-lasting (3–5 h) increases in locomotor activity (Porrino et al. 1994) and extracellular DA levels in the nucleus accumbens (Hemby et al. 1998a). Dworkin and Pitts (1994) have previously reported self-administration of WF-11 in rats reinforced under an FR10 schedule. The present experiments demonstrate that WF-11 is an extremely effective reinforcer in rats, in some cases supporting final ratio values as high as 603. Birmingham et al. (1998) have shown that WF-11 supports self-administration in monkeys reinforced under a FR schedule. Intake of WF-11 increased in a dose dependent fashion. However, the rates of responding were significantly lower than cocaine-reinforced rates which may be accounted for by rate suppressing effects (Nader et al. 1997). WF-11 blocks cocaine self-administration in rhesus monkeys for at least 3 h, and substitutes for cocaine in discrimination studies (Nader et al. 1997).

WF-23 is the prototype of the 2-naphthyl class of tropanes. WF-23 is only marginally specific (3:1) for the DA transporter compared to the 5-HT transporter; however, it is extremely potent and long acting (Daunais et al. 1998b). Contrary to the prediction that long acting drugs are less likely to be self-administered, the present

data show that WF-23 has substantial reinforcing efficacy. The striking feature of WF-23 self-administration is the long inter-infusion pauses (see Fig. 3). It should be emphasized that the present PR protocol was designed for fast acting drugs, such as cocaine (see below). Given the long duration of action of WF-23, the maximum number of injections was limited. The mean final ratios established for WF-23 at the highest dose underestimate the break point that can be obtained under different experimental conditions. Preliminary data have shown that rats self-administer WF-23 to final ratios greater than 500 when the PR series escalates quickly enough to establish a break point with 8–10 h.

WF-24 was selected for evaluation in order to test the importance of the positioning of alkyl ketone moiety at the 2-position on the tropane molecule. Note that WF-24 differs from WF-23 by having the alkyl ketone in the alpha rather than the beta position. Binding experiments have shown that this repositioning results in an approximately 20-fold reduction in affinity at the DA transporter (see Table 1). The self-administration data parallel the in vitro data. WF-24 appears to have relatively low behavioral potency and relatively limited reinforcing efficacy.

WF-31 was the prototype of a class of tropanes selective for the 5-HT transporter (Porrino et al. 1997; Daunais et al. 1998a; Hemby et al. 1998b). The concept that a phenyl isopropyl group at the 3-position yielded a 5-HT-selective compound (WF-31) directly led to the synthesis of compounds with improved 5-HT selectivity and potency, such as WF-60 (Davies et al. 1996). WF-54 and WF-55 were included in the analysis in order to address the hypothesis that adding bulk to the 3-position imparts greater 5-HT selectivity. WF-55 was found to be 3.8 times more selective at the DA transporter than at the 5-HT transporter. WF-54, which has an additional methyl added to the ethenyl at the 3-position, changed a DA transporter selective compound into a 5-HT transporter selective compound and totally eliminated the reinforcing efficacy. The present data provided no indication that the 5-HT specific compounds (WF-31, WF-54 and WF-60) had reinforcing effects at the dosages tested.

The objective of the present study was to examine tropane compounds with diverse characteristics. One dimension in which these drugs differ is duration of action. On the one hand, this allowed a systematic comparison of relatively short and long acting cocaine-like compounds. However, the present data illustrate the difficulty of using the same test parameters to examine drugs with such a wide range of durations of action.

The PR schedule can be implemented in a variety of ways. In some implementations, the ratios are incremented across days – the starting ratio on one day being dependent on performance on the previous day (Bedford et al. 1978; Griffiths et al. 1979; Risner and Goldberg 1983; Woolverton 1995). The key feature of the procedure used here is that the initial ratio is an FR1 which escalates until a break point is reached within the same session. The series of ratios was developed specifically to examine cocaine self-administration. Given the relatively short inter-infusion intervals engendered by cocaine, 16–20 injections can be self-administered within a 2- to 3-h period. A series of ratios was therefore designed to escalate so that break points (final ratios of 118–268) could be reached within a few hours. A period of 1 h without receiving an injection has typically been used to define a break point, thus a break point can be established for the most effective doses of cocaine within a 5-h session.

When testing began with the longer acting tropanes (WF-23, WF-11 and WF-55) it quickly became apparent that break points could not be established within a 5-h session. Depending on the dose, the inter-infusion intervals could be an hour or longer and as a consequence, the number of injections that could be self-administered within a session was greatly restricted. One alternative was to increase the slope of PR sequence, forcing the break point to occur earlier in the session. However, adjusting the ratio sequences for each compound would have made comparisons between drugs impossible. Instead, it was decided to hold the series constant and to extend the session length as necessary. In some cases, the break point was established within a 6-h session (e.g. WF-24), while in other cases a session length of 22 h was required.

Defining the break point was also problematic. The traditional one hour non-reinforced interval was clearly inappropriate for drugs and dosages that might normally display a very long (>1 h) inter-injection interval. In these instances, the session was extended so that at least 3 times the average inter-infusion interval had elapsed without an injection before the session was terminated (to a maximum of 22 h). Comparisons of break points between drugs must therefore be made with some caution, since fatigue and accumulated drug effects may have a greater influence over a 22-h versus a 5-h session. Since such factors might be disruptive, the present PR implementation may in fact *underestimate* the effectiveness of some of these compounds to support high break points. Notwithstanding this caveat, the present results show four of the seven selected tropane analogs supported robust self-administration behavior.

The present data also speak to the issue of duration of action and reinforcing efficacy. The most effective doses of WF-23, WF-55 and WF-11 supported breaking points values that were comparable or higher than the most effective dose of cocaine. These drugs are among the most effective reinforcers that we have examined thus far. WF-11, WF-23 and WF-55 are also relatively long acting drugs. Curiously, these data run counter to the dogma that shorter acting drugs are more likely to be reinforcing than longer acting drugs. It is unclear whether WF-11, WF-23 and WF-55 are simply exceptions to the general rule, or whether the dogma should be seriously questioned.

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